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Dated 14 May 2001

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GB0003154.2

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ASTRAZENECA AB,
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[ADP No. 07822448003]

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1. Your reference — F.2312

2. Patent application number *(The Patent Office will fill in this pa*— **0003154.2**

14FEB00 E512704-1 D02934
P01/7700 0.00-0003154.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca UK Limited
15 Stanhope Gate
LONDON
W1Y 6LN GB

SECTION 30 (1977 ACT) APPLICATION FILED 4/7/2000

Patents ADP number *(if you know it)* — 6254007002

If the applicant is a corporate body, give the country/state of its incorporation — 7810294001

4. Title of the invention — NOVEL COMPOUNDS

5. Name of your agent *(if you have one)* — Richard Summersell

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Global Intellectual Property, Patent
AstraZeneca UK Limited
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4TG
United Kingdom

Patents ADP number *(if you know it)* — 7832330001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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11. I/We request the grant of a patent on the basis of this application.

Signature Lynda M Slack Date 11 Feb 2000

Lynda M Slack, Authorised Signatory

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Lynda M Slack - 01625-516173

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NOVEL COMPOUNDS

The present invention relates to heteroaromatic carboxamides, processes and intermediates used in their preparation, pharmaceutical compositions containing them and their use in therapy.

The NF-κB (nuclear factor κB) family is composed of homo- and heterodimers of the Rel family of transcription factors. A key role of these transcription factors is to induce and co-ordinate the expression of a broad spectrum of pro-inflammatory genes including cytokines, chemokines, interferons, MHC proteins, growth factors and cell adhesion molecules (for reviews see Verma et. al., Genes Dev. 9:2723-35, 1995; Siebenlist et. al., Annu. Rev. Cell. Biol. 10;405-455 1994; Bauerle and Henkel, Ann. Rev. Immunol., 12:141-179, 1994; Barnes and Karin, New Engl. J. Med., 336;1066-1071, 1997.

The most commonly found Rel family dimer complex is composed of p50 NF□B and p65 RelA (Baeuerle and Baltimore, Cell 53:211-217, 1988; Baeuerle and Baltimore, Genes Dev. 3:1689-1698, 1989). Under resting conditions NF-κB dimers are retained in the cytoplasm by a member of the IκB family of inhibitory proteins (Beg et. al., Genes Dev., 7:2064-2070, 1993; Gilmore and Morin, Trends Genet. 9:427-433, 1993; Haskil et. al., Cell 65:1281-1289, 1991). However, upon cell activation by a variety of cytokines or other external stimuli, IκB proteins become phosphorylated on two critical serine residues (Traenckner et. al., EMBO J., 14:2876, 1995) and are then targeted for ubiquitination and proteosome-mediated degradation (Chen, Z.J. et. al., Genes and Dev. 9:1586-1597, 1995; Scherer, D.C. et. al., Proc. Natl. Acad. Sci. USA 92:11259-11263, 1996; Alkalay, I. et. al., Proc. Natl. Acad. Sci. USA 92:10599-10603, 1995). The released NF-κB is then able to translocate to the nucleus and activate gene transcription (Beg et.al., Genes Dev., 6:1899-1913, 1992).

A wide range of external stimulii have been shown to be capable of activating NF-κB (Baeuerle, P.A., and Baichwal, V.R., Adv. Immunol., 65:111-136, 1997). Although the majority of NF-κB activators result in IκB phosphorylation, it is clear that multiple pathways lead to this key event. Receptor-mediated NF-κB activation relies upon specific interactions between the receptor and adapter/signalling molecules (e.g. TRADD, RIP, TRAF, MyD88) and associated kinases (IRAK, NIK) (Song et. al., Proc. Natl. Acad. Sci. USA 94:9792-9796, 1997; Natoli et. al., JBC 272:26079-26082, 1997). Environmental

stresses such as UV light and γ-radiation appear to stimulate NF-κB via alternative, less defined, mechanisms.

Recent publications have partially elucidated the NF-κB activation. This work has identified three key enzymes which regulate specific IκB/NF-κB interactions: NF-κB inducing kinase (NIK) (Boldin et. al., Cell 85:803-815, 1996), IκB kinase-1 (IKK-1) (Didonato et. al., Nature 388:548, 1997; Regnier at. al., Cell 90:373 1997) and IκB kinase-2 (IKK-2) (Woronicz et. al., Science 278:866, 1997; Zandi et. al., Cell 91:243, 1997).

NIK appears to represent a common mediator of NF-κB signalling cascades triggered by tumour necrosis factor and interleukin-1, and is a potent inducer of IκB phosphorylation. However NIK is unable to phosphorylate IκB directly.

IKK-1 and IKK-2 are thought to lie immediately downstream of NIK and are capable of directly phosphorylating all three IκB sub-types. IKK-1 and IKK-2 are 52% identical at the amino acid level but appear have similar substrate specificity's; however, enzyme activities appear to be different: IKK-2 is several-fold more potent than IKK-1. Expression data, coupled with mutagenesis studies, suggest that IKK-1 and IKK-2 are capable of forming homo- and heterodimers through their C-terminal leucine zipper motifs, with the heterodimeric form being preferred (Mercurio et. al., Mol. Cell Biol., 19:1526, 1999; Zandi et. al., Science; 281:1360, 1998; Lee et. al, Proc. Natl. Acad. Sci. USA 95:9319, 1998).

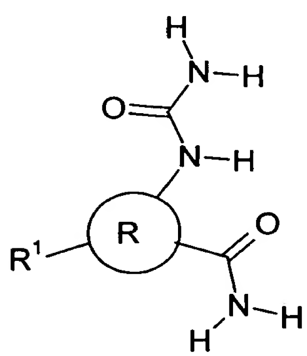
NIK, IKK-1 and IKK-2 are all serine/threonine kinases. Recent data has shown that tyrosine kinases also play a role in regulating the activation of NF-κB. A number of groups have shown that TNF-α induced NF-κB activation can be regulated by protein tyrosine phosphatases (PTPs) and tyrosine kinases (Amer et. al., JBC 273:29417-29423, 1998; Hu et. al., JBC 273:33561-33565, 1998; Kaekawa et. al., Biochem. J. 337:179-184, 1999; Singh et. al., JBC 271 31049-31054, 1996). The mechanism of action of these enzymes appears to in regulating the phosphorylation status of IκB. For example PTP1B and an unidentified tyrosine kinase appear to directly control the phosphorylation of a lysine residue (K42) on IκB-α, which in turn has a critical influence on the accessibility of the adjacent serine residues as targets for phosphorylation by IKK.

Several groups have shown that IKK-1 and IKK-2 form part of a 'signalosome' structure in association with additional proteins including IKAP, (Cohen et. al., Nature 395:292-296, 1998; Rothwarf et. al., Nature 395:297-300, 1998) MEKK-1, putative MAP kinase

phosphatase (Lee et. al., Proc. Natl. Acad. Sci. USA 95:9319-9324, 1998), as well as NIK and IκB. Data is now emerging to suggest that although both IKK-1 and IKK-2 associate with NIK, they are differentially activated, and therefore might represent an important integration point for the spectrum of signals that activate NF-κB. Importantly, MEKK-1 (one of the components of the putative signalosome and a target for UV light, LPS induced signalling molecules and small GTPases) has been found to activate IKK-2 but not IKK-1. Similarly NIK phosphorylation of IKK-1 results in a dramatic increase in IKK-1 activity but only a small effect on IKK-2 (for review see Mercurio, F., and Manning, A.M., Current Opinion in Cell Biology, 11:226-232, 1999).

Inhibition of NF-κB activation is likely to be of broad utility in treatment of inflammatory disease.

The present invention therefore provides compounds of formula (I) and pharmaceutically acceptable salts or solvates thereof:



(I)

in which:
R is a 5-membered heteroaromatic ring containing one or two heteroatoms selected from oxygen, nitrogen or sulfur;
R^1 is a phenyl group or a 5- to 7-membered heteroaromatic ring containing one to three heteroatoms selected from oxygen, nitrogen or sulfur each of which can be optionally substituted by one or more substituents selected from halogen atoms, cyano, nitro, $-OR^2$, $-NR^3R^4$, $-CONR^5R^6$, $-COOR^7$, $-NR^8COR^9$, $-SR^{10}$, $-SO_2R^{10}$, $-SO_2NR^5R^6$, $-NR^8SO_2R^{10}$, C_1-C_6 alkyl, trifluoromethyl groups or $-O(CH_2)_nR^{11}$;

each of R^2, R^3, R^4, R^5, R^6, R^7, R^8 R^8 and R^{10} independently represent a hydrogen atom or C_1-C_6, alkyl;

n is 2, 3 or 4;

R^{11} is $NR^{12}R^{13}$ where R^{12} and R^{13} are independently hydrogen or C_1-C_6, alkyl or R^{12} and R^{13} together with the nitrogen atom to which they are attached from a 5- or 6-membered saturated ring optionally containing a further O, S or NR^{14} group where R^{14} is hydrogen or C_1-C_6, alkyl;

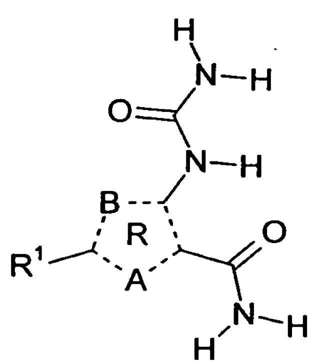
provided that:

- when R is thiophene, furan or pyrrole then R^1 is not 4-pyridinyl or 3-pyrazolyl.
- when R is oxazole, thiazole or imidazoles then R^1 is not 3-pyridinyl and 5-pyrimidyl.

In the context of the present specification, unless otherwise indicated, an alkyl or alkenyl group or an alkyl or alkenyl moiety in a substituent group may be linear or branched.

Certain compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all geometric and optical isomers of the compounds of formula (I) and mixtures thereof including racemates. Tautomers and mixtures thereof also form an aspect of the present invention.

In formula (I) the group R is a 5-membered heteroaromatic ring containing one or two heteroatoms selected from oxygen, nitrogen or sulfur. Preferably R is substituted as shown below in formula (IA) where A and B are selected from CH, S, O, NR^{11} where R^{11} is hydrogen or C_{1-6}alkyl:



(IA)

Preferred R groups include thiophene, furan, pyrrole, imidazole, thiazole and oxazole.

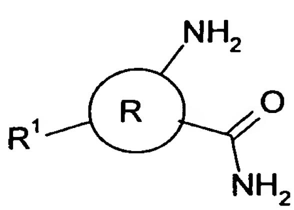
Suitably the group R^1 is a phenyl group or a 5- to 7-membered heteroaromatic ring containing one to three heteroatoms selected from oxygen, nitrogen or sulfur each of which can be optionally substituted by one or more substituents selected from halogen atoms, cyano, nitro, $-OR^2$, $-NR^3R^4$, $-CONR^5R^6$, $-COOR^7$, $-NR^8COR^9$, $-SR^{10}$, $-SO_2R^{10}$, $-SO_2NR^5R^6$, $-NR^8SO_2R^{10}$, C_1-C_6 alkyl, trifluoromethyl groups or $-O(CH_2)_nR^{11}$. When R^{11} is $NR^{12}R^{13}$ and R^{12} and R^{13} together with the nitrogen atom to which they are attached from a 5- or 6-membered saturated ring, preferred examples of such rings include morpholine, pyrrolidine and piperidine rings. When R^{11} is $NR^{12}R^{13}$ and R^{12} and R^{13} are alkyl these alkyl groups are preferably methyl.

Particularly advantageous compounds of formula (I) are those in which R^1 represents optionally substituted phenyl. More preferably R^1 represents phenyl or phenyl substituted by halogen, methoxy, hydroxy, $OCH_2CH_2NMe_2$, $OCH_2CH_2CH_2NMe_2$, morphinolylethoxy, pyrrolidinylethoxy and piperidylethoxy.

Particularly preferred compounds of the invention include those exemplified herein:
3-[(Aminocarbonyl)amino]-5-phenyl-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-chlorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(4-fluorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(4-methoxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-thienyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-hydroxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(2-chlorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(2-methoxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-methoxyphenyl)-2-thiophenecarboxamide,
2-[(Aminocarbonyl)amino]-5-phenyl-3-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,

3-[(Aminocarbonyl)amino]-5-{4-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
and pharmaceutically acceptable salts and solvates thereof.

According to the invention there is also provided a process for the preparation of a compound of formula (I) which comprises treatment of a compound of formula (II):



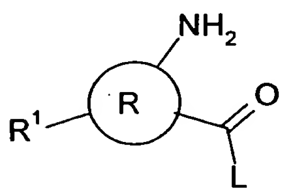
(II)

where R and R^1 are as defined in formula (I) with an isocyanate and optionally forming a pharmaceutically acceptable salt.

Suitable isocyanate reagents include trimethylsilylisocyanate, chlorosulphonylisocyanate and sodium isocyanate. The reaction with trimethylsilylisocyanate can be carried out in a

solvent such as dichloromethane/DMF at elevated temperature, for example at the reflux temperature of the reaction mixture. The reaction with chlorosulphonylisocyanate can be carried out in a solvent such as toluene at ambient temperature. The reaction with sodium isocyanate can be carried out in a suitable solvent system such as aqueous acetic acid at ambient temperature.

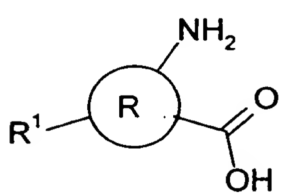
Compounds of formula (II) can be prepared by reaction of compounds of formula (III):



(III)

where R and R^1 are as defined in formula (I) with ammonia. Suitable groups L include halogen, in particular chloro.

Compounds of formula (III) where L is halo can be prepared from the corresponding compound of formula (IV):



(IV)

where R and R^1 are as defined in formula (I) by treating with a halogenating agent such as thionyl chloride.

Compounds of formula (IV) are commercially available or can be prepared using standard chemistry as exemplified herein.

It will be appreciated by those skilled in the art that in the processes of the present invention certain functional groups such as hydroxyl or amino groups in the starting

reagents or intermediate compounds may need to be protected by protecting groups. Thus, the preparation of the compounds of formula (I) may involve, at an appropriate stage, the removal of one or more protecting groups.

The protection and deprotection of functional groups is fully described in 'Protective Groups in Organic Chemistry', edited by J. W. F. McOmie, Plenum Press (1973), and 'Protective Groups in Organic Synthesis', 2nd edition, T. W. Greene & P. G. M. Wuts, Wiley–Interscience (1991).

Novel intermediate compounds form a further aspect of the invention.

The compounds of formula (I) above may be converted to a pharmaceutically acceptable salt or solvate thereof, preferably an acid addition salt such as a hydrochloride, hydrobromide, phosphate, acetate, fumarate, maleate, tartrate, citrate, oxalate, methanesulphonate or *p*-toluenesulphonate.

The compounds of formula (I) have activity as pharmaceuticals, in particular as IKK2 enzyme inhibitors, and may be used in the treatment (therapeutic or prophylactic) of conditions/diseases in human and non-human animals in which inhibition of IKK2 is beneficial. Examples of such conditions/diseases include inflammatory diseases or diseases with an inflammatory component. Specific diseases include inflammatory arthritides including rheumatoid arthritis, osteoarthritis, spondylitis, Reiters syndrome, psoriatic arthritis, lupus and bone resorptive disease; multiple sclerosis, IBD including Crohn's disease, asthma, COPD, emphysema, rhinitis, myasthenia gravis, Graves' disease, rhumatoid arthritis, allograft rejection, inflammatiry bowel disease, multiple sclerosis psoriasis, dermatitis, allergic disorders, immune complex diseases, cachexia, ARDS, toxic shock, Cardiovascular disorders, heart failure, myocardial infarcts, atherosclerosis, reperfusion injury, AIDS and cancer.

Thus, the present invention provides a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined for use in therapy.

In a further aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined in the manufacture of a medicament for use in therapy.

In a still further aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined in the manufacture of a medicament for the treatment of diseases or conditions in which modulation of the IKK2 enzyme activity is beneficial.

In the context of the present specification, the term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The terms "therapeutic" and "therapeutically" should be construed accordingly.

The invention still further provides a method of treating an IKK2 mediated disease which comprises administering to a patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined.

The invention also provides a method of treating an inflammatory disease, especially asthma, in a patient suffering from, or at risk of, said disease, which comprises administering to the patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired and the disorder indicated.

The compounds of formula (I) and pharmaceutically acceptable salts and solvates thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the formula (I) compound/salt/solvate (active ingredient) is in association with a pharmaceutically acceptable adjuvant, diluent or carrier. Depending on the mode of administration, the pharmaceutical composition will preferably comprise from 0.05 to 99 %w (per cent by weight), more preferably from 0.05 to 80 %w, still more preferably from 0.10 to 70 %w, and even more preferably from 0.10 to 50 %w, of active ingredient, all percentages by weight being based on total composition.

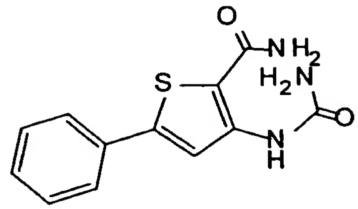
The present invention also provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

The invention further provides a process for the preparation of a pharmaceutical composition of the invention which comprises mixing a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined, with a pharmaceutically acceptable adjuvant, diluent or carrier.

The pharmaceutical compositions may be administered topically (e.g. to the lung and/or airways or to the skin) in the form of solutions, suspensions, heptafluoroalkane aerosols and dry powder formulations; or systemically, e.g. by oral administration in the form of tablets, capsules, syrups, powders or granules, or by parenteral administration in the form of solutions or suspensions, or by subcutaneous administration or by rectal administration in the form of suppositories or transdermally.

EXAMPLE 1

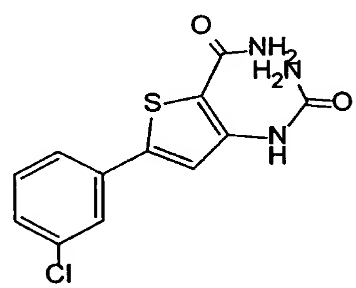
3-[(Aminocarbonyl)amino]-5-phenyl-2-thiophenecarboxamide



3-Amino-5-phenyl-2-thiophenecarboxamide ([illegible] -imethylsilylisocyanate (3mL), dichloromethane (15mL) and dimethylformamide ([illegible] were heated at reflux for 3 days. The reaction mixture was cooled and the resulting solid was filtered off, washed with methanol and then ether to give the title urea (0.39g).
m.p. >300 °C
^{1}H NMR (DMSO-D6) 10.06(1H, s); 8.25(1H,s); 7.62 (2H, d); 7.50-7.37 (5H, m); 6.63 (2H, s).

EXAMPLE 2

3-[(Aminocarbonyl)amino]-5-(3-chlorophenyl)-2-thiophenecarboxamide



a) Methyl 3-amino-5-(3-chlorophenyl)-2-thiophenecarboxylate
Phosphorous oxychloride (6.7mL) was added to dimethylformamide (11mL) with ice cooling to keep the internal temperature below 25°. After 20 minutes, (3-chlorophenyl)ethanone (5g) was added portionwise keeping the internal temperature below 30°. The reaction mixture was heated to 50° and then treated cautiously with hydroxylamine hydrochloride (10g). The reaction mixture was stirred for 20 minutes at room temperature and water (50mL) was added. After a further 30 minutes, the reaction

mixture was extracted three times with ethyl acetate. The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated to give an oil. This oil was dissolved in methanol (50mL) and treated with methyl mercaptoacetate (2.7mL) and sodium methoxide (7.3mL of 25% in methanol). After reflux for one hour, the cooled reaction mixture was reduced to one third volume and water was added. The reaction mixture was extracted three times with ethyl acetate. The combined extracts were dried ($MgSO_4$), the solvent was evaporated and the residue was chromatographed on silica eluting with dichloromethane/isohexane mixtures to give the sub-title ester (2.0g).

m.p. 105-6 °C

MS (EI) 267 $(M)^+$

^{1}H NMR (DMSO-D6) 7.68 (1H, s); 7.60 (1H, m); 7.48 (2H, m); 7.02 (1H, s); 6.60 (2H, s); 3.74 (3H, s).

b) 3-Amino-5-(3-chlorophenyl)-2-thiophenecarboxylic acid

Methyl 3-amino-5-(3-chlorophenyl)-2-thiophenecarboxylate (1.0g,), sodium hydroxide (2mL of 2M) and methanol (10 mL) were heated at 70° for 2 days. The methanol was evaporated and the residue was acidified with hydrochloric acid (2M). Extraction into ethyl acetate followed by drying ($MgSO_4$) and evaporation of the solvent gave the sub-title acid (0.8g).

MS (APCI) 252 $(M+H)^+$

^{1}H NMR (DMSO-D6) 7.62 (1H, d); 7.60 (1H, m); 7.43 (2H, m); 7.02 (1H, s); NH_2 and COOH protons not observed.

c) 3-Amino-5-(3-chlorophenyl)-2-thiophenecarboxamide

3-Amino-5-(3-chlorophenyl)-2-thiophenecarboxylic acid (0.8g,) and thionyl chloride (20 mL) were heated at reflux for one hour. After cooling, the excess thionyl chloride was evaporated, final traces were removed by azeotroping with toluene. The residue was dissolved in acetonitrile (50 mL) and ammonia (d=0.88, 10mL) was added. After stirring for one hour, the solvent was evaporated and the residue chromatographed on silica eluting with ethyl acetate/dichloromethane mixtures. Trituration with ether gave the sub-title amide (0.48g).

m.p. 164-5 °C

MS (APCI) 253 $(M+H)^+$

^{1}H NMR (DMSO-D6) 7.62 (1H, d); 7.55 (1H, dd); 7.45 (2H, m); 7.02 (1H, s);6.98 (2H, s); 6.50 (2H, s).

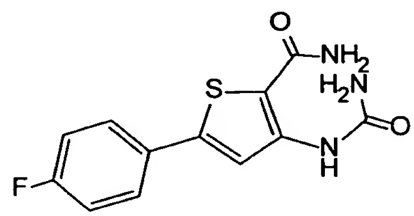
d) 3-[(Aminocarbonyl)amino]-5-(3-chlorophenyl)-2-thiophenecarboxamide

Prepared by the method of Example 1 from 3-amino-5-(3-chlorophenyl)-2-thiophenecarboxamide and trimethylsilylisocyanate.

m.p. >300 °C
MS (APCI) 253 $(M+H)^+$
^{1}H NMR (DMSO-D6) 10.03 (1H, s); 8.30 (1H, s); 7.62 (1H, d); 7.60-7.40 (4H, m); 7.30-7.00 (1H, m); 6.70 (2H, s).

EXAMPLE 3

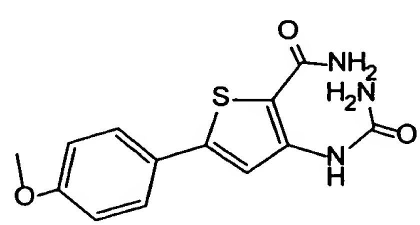
3-[(Aminocarbonyl)amino]-5-(4-fluorophenyl)-2-thiophenecarboxamide



Sodium cyanate (1.08g) was added portionwise to a stirred suspension of 3-amino-5-(4-fluorophenyl)-2-thiophenecarboxamide (3.2g) in acetic acid (150mL) and water (90mL). After 20 hours, the solid was filtered off and washed with water, methanol and ether. Recrystallisation from methanol/dimethylsulphoxide gave the title urea (0.5g) as a 1:1 dimethylsulphoxide solvate.
m.p. >320 °C
MS (APCI) 278 $(M-H)^+$
^{1}H NMR (DMSO-D6) 10.07 (1H, s); 8.22 (1H, s); 7.67 (2H, t); 7.40 (2H, s); 7.29 (2H, t); 6.65 (2H, s).

EXAMPLE 4
3-[(Aminocarbonyl)amino]-5-(4-methoxyphenyl)-2-thiophenecarboxamide

Prepared by the method of example 1 from 3-amino-5-(4-methoxyphenyl)-2-

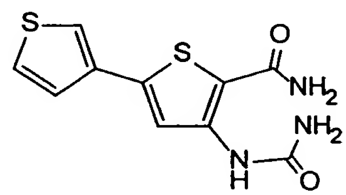


thiophenecarboxamide and trimethylsilylisocyanate.
m.p. >300 °C

MS (APCI) 292 $(M+H)^+$
^{1}H NMR (DMSO-D6) 10.06 (1H, s); 8.12 (1H, s); 7.55 (2H, d); 7.37 (2H, s); 7.03 (2H, d); 6.61 (2H, s); 3.80 (3H, s).

EXAMPLE 5

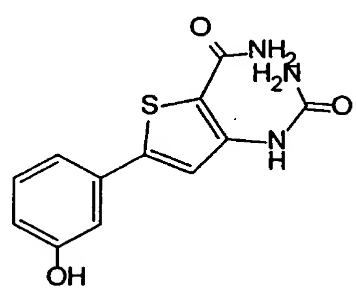
3-[(Aminocarbonyl)amino]-5-(3-thienyl)-2-thiophenecarboxamide



Prepared by the method of example 1 from 3-amino-5-(3-thienyl)-2-thiophenecarboxamide and trimethylsilylisocyanate.
NMR

EXAMPLE 6

3-[(Aminocarbonyl)amino]-5-(3-hydroxyphenyl)-2-thiophenecarboxamide

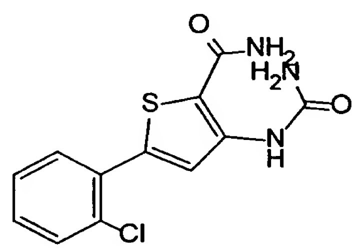


3-Amino-5-(3-methoxyphenyl)-2-thiophenecarboxamide (0.5g,), trimethylsilylisocyanate (2mL), dimethylformamide (2mL) and dichloromethane were heated at reflux for 3 days. After cooling the solid was filtered off, suspended in dichloromethane (100mL) and treated with boron tribromide (5mL of 1M in dichloromethane). After 3 days, methanol (50mL) was added. After one hour, the solvent was evaporated and the residue was titurated with hydrochloric acid (2M). The title urea was filtered off (0.35g).
m.p. >300 °C
MS (APCI) 278 $(M+H)^+$

^1H NMR (DMSO-D6) 10.05 (1H, s); 9.71 (1H, s); 8.19 (1H, s); 7.41 (2H, m); 7.26 (1H, t); 7.03 (2H, m); 6.79 (1H, dd); 6.62 (2H, s).

EXAMPLE 7

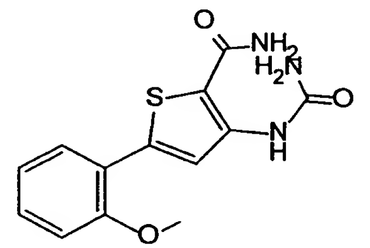
3-[(Aminocarbonyl)amino]-5-(2-chlorophenyl)-2-thiophenecarboxamide AR-C141817



a) 3-Amino-5-(2-chlorophenyl)-2-thiophenecarboxylic acid
Prepared by the method of example 2b) from methyl 3-amino-5-(2-chlorophenyl)-2-thiophenecarboxylate.
MS (APCI) 252 $(M+H)^+$
^1H NMR (DMSO-D6) 7.60 (2H, m); 7.40 (2H, m); 6.92 (1H, s); NH_2 and COOH protons not observed.
b) 3-Amino-5-(2-chlorophenyl)-2-thiophenecarboxamide
Prepared by the method of example 2c) from 3-amino-5-(2-chlorophenyl)-2-thiophenecarboxylic acid.
m.p. 87-9 °C
MS (APCI) 253 $(M+H)^+$
^1H NMR (DMSO-D6) 7.60 (2H, m); 7.40 (2H, m); 7.00 (2H, s); 6.90 (1H, s); 6.42 (2H, s).
c) 3-[(Aminocarbonyl)amino]-5-(2-chlorophenyl)-2-thiophenecarboxamide
Prepared by the method of Example 1 from 3-amino-5-(2-chlorophenyl)-2-thiophenecarboxamide and trimethylsilylisocyanate.
m.p. >300 °C
MS (APCI) 296 $(M+H)^+$
^1H NMR (DMSO-D6) 7.34 (2H, s); 6.80 (2H, m); 6.70 (2H, m); 6.52 (4H, m).

EXAMPLE 8

3-[(Aminocarbonyl)amino]-5-(2-methoxyphenyl)-2-thiophenecarboxamide



a) Methyl 3-amino-5-(2-methoxyphenyl)-2-thiophenecarboxylate

Prepared by the method of example 2a) from (2-methoxyphenyl)ethanone.

m.p. 119-20 °C

MS (APCI) 264 $(M+H)^+$

^{1}H NMR (DMSO-D6) 7.62 (1H, dd); 7.40 (1H, t); 7.18 (1H, d); 7.05 (1H, s); 7.02 (1H, t); 6.45 (2H, s); 3.95 (3H, s); 3.75 (3H, s).

b) 3-Amino-5-(2-methoxyphenyl)-2-thiophenecarboxylic acid

Prepared by the method of example 2b) from methyl 3-amino-5-(2-methoxyphenyl)-2-thiophenecarboxylate and used directly for step c).

c) 3-Amino-5-(2-methoxyphenyl)-2-thiophenecarboxamide

Prepared by the method of example 2c) from 3-amino-5-(2-methoxyphenyl)-2-thiophenecarboxylic acid and used directly for step d).

d) 3-[(Aminocarbonyl)amino]-5-(2-methoxyphenyl)-2-thiophenecarboxamide

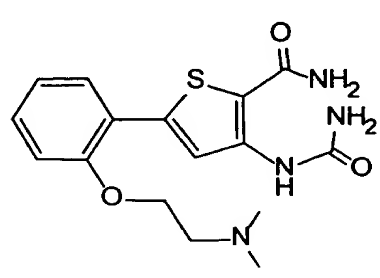
Prepared by the method of Example 1 from 3-amino-5-(2-methoxyphenyl)-2-thiophenecarboxamide and trimethylsilylisocyanate.

m.p. >300 °C

^{1}H NMR (DMSO-D6) 10.01 (1H, s); 8.33 (1H, s); 7.62 (1H, dd); 7.40-7.00 (5H, m); 6.57 (2H, s); 3.90 (3H, s).

EXAMPLE 9

3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide



a) 3-[(Aminocarbonyl)amino]-5-(2-hydroxyphenyl)-2-thiophenecarboxamide

3-[(Aminocarbonyl)amino]-5-(2-methoxyphenyl)-2-thiophenecarboxamide (0.1g), boron tribromide (2ml of 1M in dichloromethane) and dichloromethane (10 mL) were stirred at room temperature for 16 hours. Methanol (5 mL) was added and after one hour the solvent was evaporated. Hydrochloric acid (10mL of 2M) was added and, after stirring for one hour, the phenol was filtered off. Used directly in step b).

b) 3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide

The phenol (0.05g), potassium carbonate (0.05g) and (2-chloroethyl)dimethylamine hydrochloride (0.03g) in dimethylformamide (2mL) were stirred at 80° for 24 hours. The cooled reaction was poured onto ethyl acetate and brine. The aqueous layer was separated and washed with twice with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and the solvent was evaporated. Chromatography on silica eluting with dichloromethane/methanol mixtures gave the title compound (6mg).

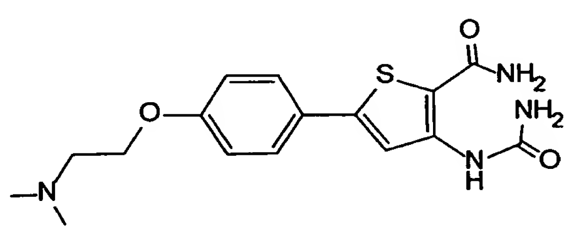
m.p. 180 °C

MS (APCI) 349 $(M+H)^+$

^{1}H NMR (DMSO-D6) 10.00 (1H, s); 8.40 (1H, s); 7.62 (1H, dd); 7.38 (3H, m); 7.20 (1H, d); 7.05 (1H, t); 6.60 (2H, s); 4.20 (2H, t); 2.80 (2H, t); 2.50 (6H,s).

EXAMPLE 10

3-[(Aminocarbonyl)amino]-5-{4-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide



a) 3-[(Aminocarbonyl)amino]-5-(4-hydroxyphenyl)-2-thiophenecarboxamide

Prepared by the method of example 6 from 3-amino-5-(4-methoxyphenyl)-2-thiophenecarboxamide and used directly in step b).

b) 3-[(Aminocarbonyl)amino]-5-{4-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide

Prepared by the method of example 9b) from 3-[(aminocarbonyl)amino]-5-(4-hydroxyphenyl)-2-thiophenecarboxamide.

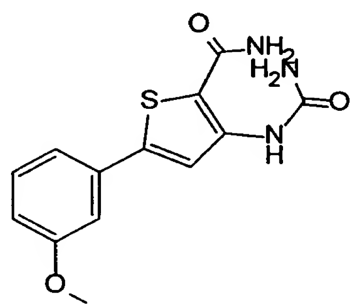
m.p. >300 °C

MS (APCI) 349 $(M+H)^+$

^1H NMR (DMSO-D6) 10.06 (1H, s); 8.12 (1H, s); 7.53 (2H, d); 7.40 (2H, s); 7.00 (2H, d); 6.60 (2H, s); 4.10 (2H, t); 2.60 (2H, t); 2.20 (6H, s).

EXAMPLE 11

3-[(Aminocarbonyl)amino]-5-(3-methoxyphenyl)-2-thiophenecarboxamide



a) Methyl 3-amino-5-(3-methoxyphenyl)-2-thiophenecarboxylate

Prepared by the method of example 2a) from (3-methoxyphenyl)ethanone.

m.p. 81-2 °C

MS (APCI) 264 $(M+H)^+$

^1H NMR (DMSO-D6) 7.40 (1H, t); 7.20 (1H, d); 7.15 (1H, m); 7.00 (2H, m); 6.60 (2H, s); 3.80 (3H, s); 3.70 (3H, s).

b) 3-Amino-5-(3-methoxyphenyl)-2-thiophenecarboxylic acid

Prepared by the method of example 2b) from methyl 3-amino-5-(3-methoxyphenyl)-2-thiophenecarboxylate and used directly in step c).

c) 3-Amino-5-(3-methoxyphenyl)-2-thiophenecarboxamide

Prepared by the method of example 2c) from 3-amino-5-(3-methoxyphenyl)-2-thiophenecarboxylic acid.

m.p. 101-3 °C

MS (APCI) 249 $(M+H)^+$

^1H NMR (DMSO-D6) 7.35 (1H, t); 7.20 (1H, d); 7.10 (1H, m); 7.00-6.90 (4H, m); 6.42 (2H, s); 3.80 (3H, s).

d) 3-[(Aminocarbonyl)amino]-5-(3-methoxyphenyl)-2-thiophenecarboxamide

Prepared by the method of Example 1 from 3-amino-5-(3-methoxyphenyl)-2-thiophenecarboxamide and trimethylsilylisocyanate.

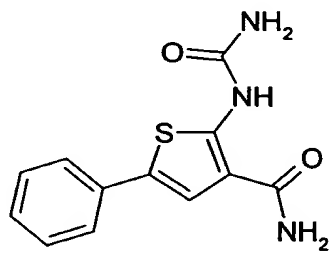
m.p. 105-6 °C

MS (EI) 267 $(M)^+$

^{1}H NMR (DMSO-D6) 10.05 (1H, s); 8.23 (1H, s), 7.43 (2H, s); 7.39 (1H, t); 7.19 (1H, d); 7.10 (1H, s); 6.98 (1H, d); 6.62 (2H, s); 3.82 (3H, s).

EXAMPLE 12

2-[(Aminocarbonyl)amino]-5-phenyl-3-thiophenecarboxamide



Chlorosulphonylisocyanate (0.081mL) was added to a stirred suspension at 0°C of 2-amino-5-phenyl-3-thiophenecarboxamide (0.2g) in toluene (10mL). After stirring for 16 hours at room temperature, the solvent was evaporated and the residue dissolved in acetonitrile (20mL). Sodium bicarbonate solution (2mL of 10%) was added and the mixture was stirred for one hour. After acidification with 2M hydrochloric acid, the solution was extracted three times with ethyl acetate. The combined extracts were dried ($MgSO_4$) and the solvent was evaporated. Chromatography on silica eluting with methanol/dichloromethane mixtures gave the title urea (0.027g).

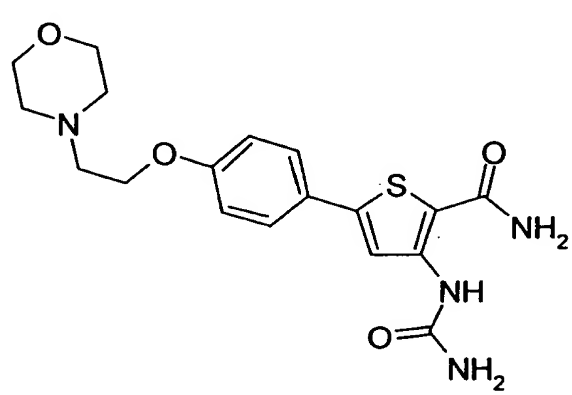
m.p. 395 °C

MS (APCI) 262 $(M+H)^+$

^{1}H NMR (DMSO-D6) 11.01 (1H, s); 7.73 (1H, s); 7.69 (1H, s); 7.58 (1H, s); 7.54 (1H, s); 7.40 (2H, t); 7.35-7.20 (2H, m); 7.00 (2H, s).

EXAMPLE 13

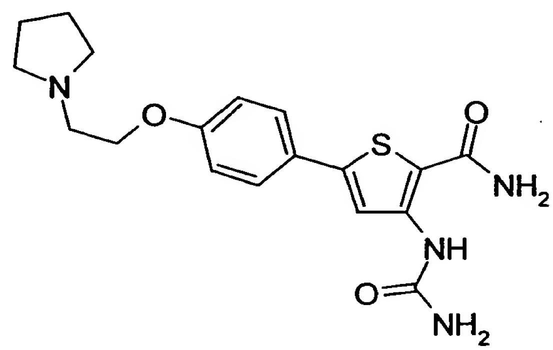
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (EI) 390 $(M)^+$.

EXAMPLE 14

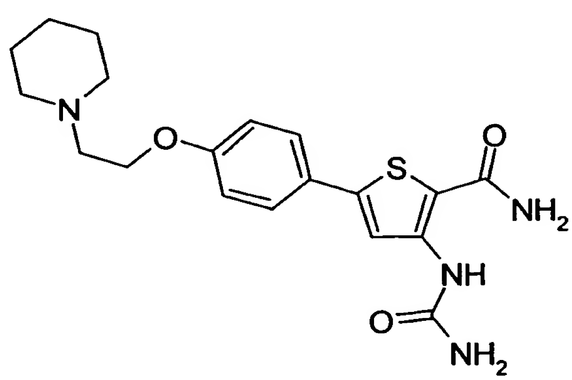
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (EI) 374 $(M)^+$

EXAMPLE 15

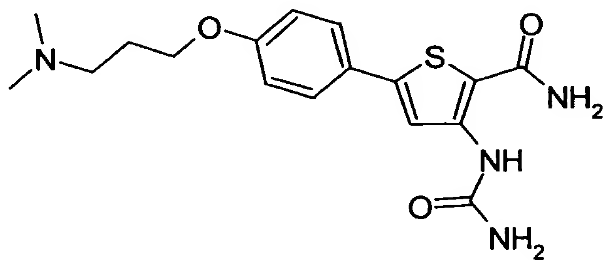
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (EI) 388 $(M)^+$

EXAMPLE 16

3-[(Aminocarbonyl)amino]-5-{4-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide

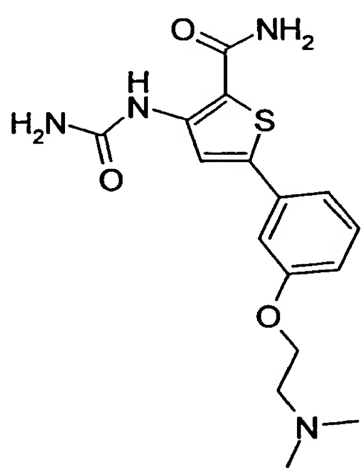


Prepared by the method of example 9b).

MS (EI) 362 $(M)^+$

EXAMPLE 17

3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide

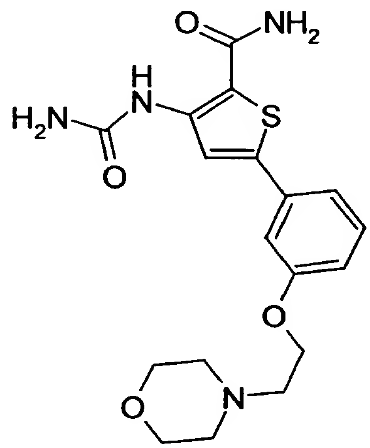


Prepared by the method of example 9b).

MS (EI) 348 $(M)^+$

EXAMPLE 18

3-[(Aminocarbonyl)amino]-5-{3-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide

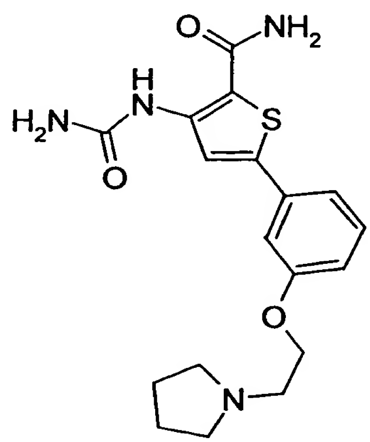


Prepared by the method of example 9b).

MS (EI) 390 $(M)^+$

EXAMPLE 19

3-[(Aminocarbonyl)amino]-5-{3-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide

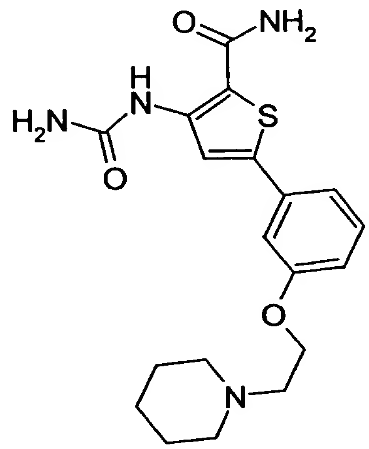


Prepared by the method of example 9b).

MS (EI) 374 $(M)^+$

EXAMPLE 20

3-[(Aminocarbonyl)amino]-5-{3-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide

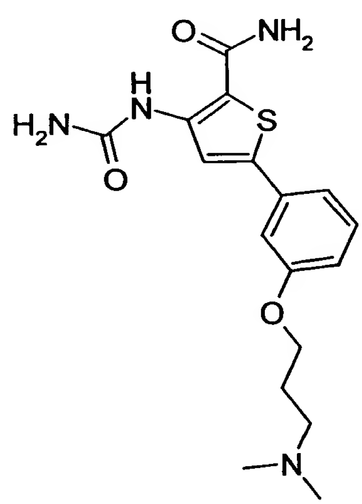


Prepared by the method of example 9b).

MS (EI) 388 $(M)^+$

EXAMPLE 21

3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide

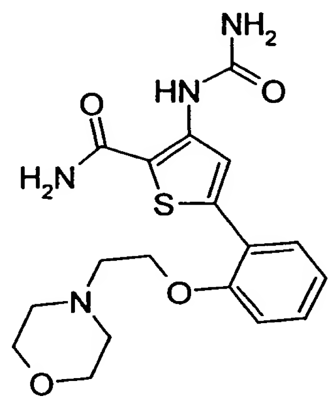


Prepared by the method of example 9b).

MS (EI) 362 $(M)^+$

EXAMPLE 22

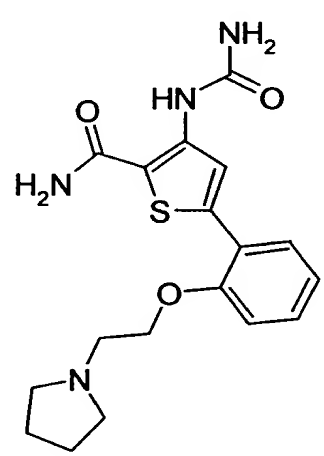
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (APCI) 391 $(M+H)^+$

EXAMPLE 23

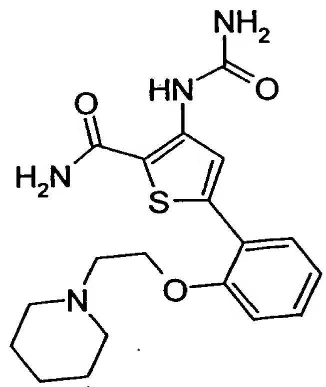
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (APCI) 375 $(M+H)^+$

EXAMPLE 24

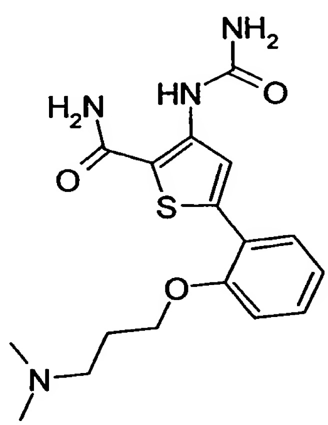
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (APCI) 389 $(M+H)^+$

EXAMPLE 25

3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide



Prepared by the method of example 9b).
MS (APCI) 363 $(M+H)^+$

Pharmacological Data

1. PBMC/Compound Assay

Plating Blood

250ml Blood was ordered from CPU.

25ml blood was layered on 20ml Lymphoprep (Nycomed) in 50ml Falcon Tube.

Tubes were centrifuged at 2.5krpm (in Sorval RT600B) for 30min.

Cloudy PBMC layer was collected with a fine tipped Pasteur pipette into clean falcon tubes (about 10ml) and made up to 50ml with PBS (10 tubes were combined into 8 tubes).

Tubes were centrifuged at 2k rpm for 8min.

10ml PBS was added to pellets and the cells were re-suspended by gently but repeatedly pipetting with 10ml stripette. 8 tubes were combined into 4 tubes. Volume in each tube was made up to 50ml with PBS.

Tubes were centrifuged at 1.4k rpm for 8min.

Pellets were re-suspended as before and combined into 2 tubes. Volume was made up to 50ml with PBS.

Tubes were centrifuged at 0.9k rpm for 10min.

Pellets were re-suspended in 10ml media (RPMI, 1% HI (heat-inactivated: 56°C, 60 min) human serum, L-glu and pen-strep) and combined into 1 tube, an additional 10ml media was used to rinse round discarded tube and was added to cells ie all cells in 1 tube in 30ml media.

Cell count was performed and the cell suspension was diluted to 2.6×10^6/ml. 100µl/well was then plated to the middle 60 wells and 3 middle wells of bottom row of a 96 well plate.

200µl/well PBS was added to the other outside wells to reduce evaporation. See plate layout below. Duplicate plates were made if 3H-Leucine assay was to be performed.

					PBS						
	Compound 1			Compound 2			Compound 3				
										Min	
											PBS
PBS											
										Max	
		PBS		10µM M321977					PBS		

Compounds

Compounds were solubilised in DMSO by the robot to 10mM and diluted 1:250 by hand to 40µM into RPMI medium. (1.2µl of compound was added to 300µl medium) in triplicate wells in row B of a 96 well plate using a fresh pipet for each well.

1:3 serial dilutions (100µl into 200µl) were then performed down to row G in 0.4% DMSO RPMI media

50µl/well was transferred to the cells, into a final volume of 200µl, such that the final concentrations tested were 10, 3.33, 1.11, 0.31, 0.12 and 0.04µM

Cells were then incubated for 30min before stimulation with LPS (Sigma/L-4130) (50µl/well at 4µg/ml (=1ug/ml final)) diluted from frozen stock at 5mg/ml. Cells were then incubated overnight.

Min controls = 0.4% DMSO media instead of compound. 50µl media instead of LPS.
Max controls = 0.4% DMSO media instead of compound. LPS stimulated as normal.
M321977 control = 50µl 10µM m321977 added for compound, then LPS stimulated. This was used as an additional control

3H-Leucine

20μl per well RPMI media containing 1μCi 3H-Leucine(Amersham/TRK 754/63Ci/mmol) (0.2μl at 5μCi/μl) was added to the cells which were then incubated overnight.

Plates were filtered using a Tomtec cell harvester and the filtermats dried overnight at 37°C.

Filter mats were sealed in plastic bags containing 5ml scintillant and counted.

WST-1

Plates were centrifuged for 4 minutes at 1,200rpm before supernatants were removed. Whole rows were removed at a time using a multichannle, from top to bottom of the plate, with tips changed for each plate and stored at -20°C, for TNF ELISA

100μl per well RPMI media containing 10μl WST-1 reagent (Boehringer mannheim/1044807)was added to the cells.

The cells were then incubated for 0.5-3hr.

Plates were then read using the Spectramax 250 at 450nm.

TNF ELISA

Wells were coated overnight at 4°C with 100μl, 1μg/ml sheep anti hTNFα monoclonal antibody (in house: contact Robert Forder) diluted in coating buffer. (eg 27.5μl 2mg/ml stock in 55ml for 7 plates). Blank wells (see below) were not coated.

Wells were washed once with 0.1%(w/v) BSA PBS-tween (w/v -0.05%)

Wells were then blocked for 1hr at room temperature with 200μl 1% BSA in coating buffer.

Wells were washed 3 times with 0.1% BSA PBS-tween (0.05%)

100µl/well sample (cell supernatant removed for WST-1 assay diluted 1:3 with 1% BSA PBS-tween) or standard (10, 3.3, 1.1, 0.37, 0.12, 0.04, 0.014 and 0 ng/ml) was incubated at room temperature for 2 hours. Standards were made by diluting 6µl 5µg/ml stock in 3ml dilution buffer, then performing serial 1:3 dilutions, 1ml into 2ml.

					Blank						
	Compound 1			Compound 2			Compound 3				
										Min	
std											std
										Max	
				10µM M321977							

Wells were washed 3 times with 0.1% BSA PBS-tween (0.05%)

100µl/well 2.5µg/ml rabbit anti hTNFα (in house Ab) was incubated at room temperature for 1.5 hr. (eg 38.1µl 4mg/ml stock diluted in 61ml for 7 plates)

Wells were washed 3 times with 0.1% BSA PBS-tween (0.05%)

100µl/well goat anti rabbit HRP conjugate (ICN/674371) diluted 1/10 000 from stock) was incubated at room temperature for 1.5hr eg 6.1µl in 61ml for 7 plates.

Wells were washed 3 times with 0.1% BSA PBS-tween (0.05%)

100µl substrate (1mg TMB tablet (Sigma/T-5525)dissolved in 100µl DMSO, added to 10ml citrate acetate buffer, with 36µl UHPO (BDH/30559 1g tablet dissolved in 25ml distilled water) for 1 plate) was then added to each well and incubated, in the dark, at room temperature for 30min.

The reaction was stopped by adding 25µl 2M H_2SO_4 to each well

Plates were then read at 450nm using a spectramax 250

Coating buffer = 0.5M carbonate/bicarbonate buffer.
1.59g sodium carbonate, 2.93g sodium bicarbonate, 0.2g sodium azide, made up to 1l in distilled water and pH checked (pH9.6)

Substrate buffer = 0.1M sodium acetate/citrate
13.6g sodium acetate (trihydrate salt) in ~ 900ml distilled water. pH to pH6 with 0.1M Citric acid.

2. IKK1 Filter Kinase Assay

Test Compound Dilution

The test compounds were dissolved to 10 mM in DMSO, the compounds were then diluted 1 in 40 in kinase buffer (15ul Compound + 585ul buffer). This dilution was made into row A of a 96 deep well plate in duplicate (5 compounds per plate). The Tecan Robot carried out the dissolving and diluting of compounds up to this point, the rest was carried out by hand.

150ul of each compound was transfered in duplicate to row A of a 96-well plate. Each compound was then serially diluted 1 in 3 (50ul compound + 100ul buffer) seven times in to 2.5 % DMSO/Kinase buffer to , 83.33, 27.78, 9.26, 3.09, 1.03, and 0.34 μM. 20 ul of compound dilution was added to a 96 well plate in duplicate. The final assay volume was 50 ul so that the final test concentrations were 100, 33.33, 11.11, 3.70, 1.23, 0.41, 0.14, and 0.05μM and the final DMSO concentration was 1 %.

Controls

20 ul 2.5% DMSO/Kinase Bufffer was added to each well in the 11th column of each plate to demonstrate the maximum cpms possible . 20 ul 0.5 M EDTA was added to each well of the 12th column of each plate to demonstrate the minimum cpms possible i.e the background.

ATP Mix

A mixture of unlabelled ATP, MgOAc and Labelled ATP was made, such that each well contained 10 mM MgOAc, 1 uM ATP and 0.1 uCi ^{33}P ATP. 10 ul of ATP mix was added to each well.

	ul/well	ul/plate	ul/assay (7plts)
100 mM MgOAc	5	500	3500
1 mM ATP	0.05	5	35
Kinase Buffer	4.75	475	63465
^{33}P γ ATP 10 uCi/ul	0.01	1	7

Kinase Mix

The kinase mix was made up to include 0.25 ug/well IKK1 with 9 ug/well 1-53 GST-IKB, and kinase buffer to a total volume of 20 ul/well. 20 ul of kinase mix was added to each well to start the reaction. For example:

IKK1	ul/well	ul/plate	ul/assay (7 plates)
IKK1 0.55 mg/ml	0.454	45.4	318
IKB 2.85 mg/ml	3.06	306	2142
Hepes	6.94	694	4858
Kinase Buffer	9.55	955	6682

The kinase reactions were incubated at 21°C for 80 min and the reaction stopped by precipitating the protein by the addition of an equal volume (50ul) of 20 % TCA. The precipitate was allowed to form for 10 min and then filtered through a Packard Unifilter onto GF/C unifilter 96 well plates. Each well was washed twice with approximately 1ml 2 % TCAand either left on the bench overnight to dry or dried at 30- 40°C for 60 min. 20ul microscint 40 was added to each well, the plates sealed and counted on a Packard Topcount microplate scintilation counter.

Buffers

Kinase Buffer	**1 Litre**
50 mM Tris pH 7.4	7.58g Trizma Pre-set

	crystals
0.1 mM EGTA	38.04 mg
0.1mM sodium Orthovanadate	1 ml of 0.1 M stock
0.1 % β-mercaptoethnol (added on day of assay)	1ul/ml added fresh
dd H_2O	to 1000 ml

0.1mM Sodium Orthovanadate	**100ml**
Sodium Orthovanadate	1.84g
dd H_2O	to 100 ml

HEPES Buffer	**100 ml**
20 mM Hepes 92-ethanesulphonic acid)	0.477g
2mM $MgCl_2$	2 ml 100mM stock
2mM $MnCl_2$	2 ml 100mM stock
dd H_2O	to 100ml

100 mM MgoAc

Magnesium acetate		2.145g
dd H_2O	to 100 ml	

1 mM ATP

1. Make 100mM solution if ATP by dissolving 55.11g ATP in 1 ml dd H_2O.
2. Adjust pH to 6.8 by adding drops of 5M NaOH, check by spotting on to narrow range pH paper. Mix rapidly betwwen additions because ATP is labile in alkali.
3. Make 1 in 4000 dilution and read absorbance at 259 nm. Given that absorbance at 259 nm of 1mM ATP = 15.4 calculate actual concentration:

CONCENTRATION (MG/ML) = OD AT 259 NM X 4000 / 15.4

4. Using calculated concentration dilute to 1 mM, make 1 in 40 dilution and re-OD to check.

3. IKK2 Filter Kinase Assay

Test Compound Dilution

The test compounds were dissolved to 10 mM in DMSO, the compounds were then diluted 1 in 40 in kinase buffer (15ul Compound + 585ul buffer). This dilution was made into row A of a 96 deep well plate in duplicate (5 compounds per plate). The Tecan Robot carried out the dissolving and diluting of compounds up to this point, the rest was carried out by hand.

150ul of each compound was transfered in duplicate to row A of a 96-well plate. Each compound was then serially diluted 1 in 3 (50ul compound + 100ul buffer) seven times in to 2.5 % DMSO/Kinase buffer to , 83.33, 27.78, 9.26, 3.09, 1.03, and 0.34 μM. 20 ul of compound dilution was added to a 96 well plate in duplicate. The final assay volume was 50 ul so that the final test concentrations were 100, 33.33, 11.11, 3.70, 1.23, 0.41, 0.14, and 0.05μM and the final DMSO concentration was 1 %.

Controls

20 ul 2.5% DMSO/Kinase Bufffer was added to each well in the 11th column of each plate to demonstrate the maximum cpms possible . 20 ul 0.5 M EDTA was added to each well of the 12th column of each plate to demonstrate the minimum cpms possible i.e the background.

ATP Mix

A mixture of unlabelled ATP, MgOAc and Labelled ATP was made, such that each well contained 10 mM MgOAc, 1 uM ATP and 0.1 uCi ^{33}P ATP. 10 ul of ATP mix was added to each well.

	ul/well	ul/plate	ul/assay (7plts)
100 mM MgOAc	5	500	3500
1 mM ATP	0.05	5	35
Kinase Buffer	4.75	475	3465
^{33}P γ ATP 10 uCi/ul	0.01	1	7

Kinase Mix

The kinase mix was made up to include 0.15 ug/well IKK2 with 0.5 ug /well 1-53 GST-IKB, 8.5 ug BSA (to aid precipitation of protein) and kinase buffer to a total volume of 20 ul/well. 20 ul of kinase mix was added to each well to start the reaction. For example:

IKK2	ul/well	ul/plate	ul/assay (7 plates)
IKK2 0.46 mg/ml	0.326	32.6	228
IKB 2.85 mg/ml	0.165	16.5	115
BSA 10 mg/ml	0.85	85	595
Hepes	8.99	899	6293
Kinase Buffer	9.67	967	6769

The kinase reactions were incubated at 21°C for 80 min and the reaction stopped by precipitating the protein by the addition of an equal volume (50ul) of 20 % TCA. The precipitate was allowed to form for 10 min and then filtered through a Packard Unifilter onto GF/C unifilter 96 well plates. Each well was washed twice with approximately 1ml 2 % TCAand either left on the bench overnight to dry or dried at 30- 40°C for 60 min. 20ul microscint 40 was added to each well, the plates sealed and counted on a Packard Topcount microplate scintilation counter.

Buffers

Kinase Buffer	**1 Litre**
50 mM Tris pH 7.4	7.58g Trizma Pre-set crystals
0.1 mM EGTA	38.04 mg
0.1mM sodium Orthovanadate	1 ml of 0.1 M stock
0.1 % β-mercaptoethnol (added on day of assay)	1ul/ml added fresh
water	to 1000 ml

0.1mM Sodium Orthovanadate	**100ml**
Sodium Orthovanadate	1.84g
dd H_2O	to 100 ml

HEPES Buffer	**100 ml**
20 mM Hepes 92-ethanesulphonic acid)	0.477g
2mM $MgCl_2$	2 ml 100mM stock
2mM $MnCl_2$	2 ml 100mM stock
dd Water	to 100ml

100 mM MgoAc

Magnesium acetate	2.145g
dd Water	to 100 ml

1 mM ATP

1. Make 100mM solution if ATP by dissolving 55.11g ATP in 1 ml dd H_2O.
2. Adjust pH to 6.8 by adding drops of 5M NaOH, check by spotting on to narrow range pH paper. Mix rapidly betwwen additions because ATP is labile in alkali.

3. Make 1 in 4000 dilution and read absorbance at 259 nm. Given that absorbance at 259 nm of 1mM ATP = 15.4 calculate actual concentration:

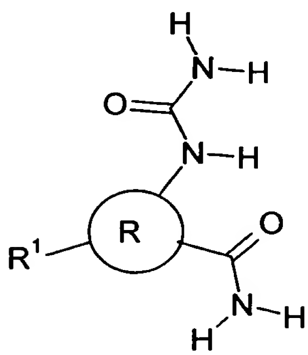
CONCENTRATION (MG/ML) = OD AT 259 NM X 4000 / 15.4

4. Using calculated concentration dilute to 1 mM, make 1 in 40 dilution and re-OD to check.

The compounds exemplified are active at less than 10 μM in the IKK2 screen.

CLAIMS

1. A compounds of formula (I) and pharmaceutically acceptable salts or solvates thereof:



(I)

in which:

R is a 5-membered heteroaromatic ring containing one or two heteroatoms selected from oxygen, nitrogen or sulfur;

R^1 is a phenyl group or a 5- to 7-membered heteroaromatic ring containing one to three heteroatoms selected from oxygen, nitrogen or sulfur each of which can be optionally substituted by one or more substituents selected from halogen atoms, cyano, nitro, $-OR^2$, $-NR^3R^4$, $-CONR^5R^6$, $-COOR^7$, $-NR^8COR^9$, $-SR^{10}$, $-SO_2R^{10}$, $-SO_2NR^5R^6$, $-NR^8SO_2R^{10}$, C_1-C_6 alkyl, trifluoromethyl groups or $-O(CH_2)_nR^{11}$;

each of R^2, R^3, R^4, R^5, R^6, R^7, R^8 R^8 and R^{10} independently represent a hydrogen atom or C_1-C_6, alkyl;

n is 2, 3 or 4; and

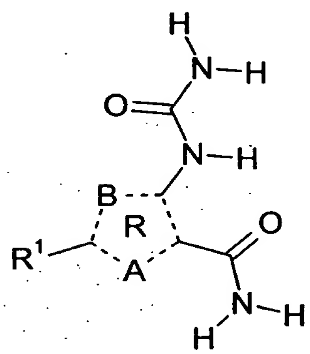
R^{11} is $NR^{12}R^{13}$ where R^{12} and R^{13} are independently hydrogen or C_1-C_6, alkyl or R^{12} and R^{13} together with the nitrogen atom to which they are attached from a 5- or 6-membered saturated ring optionally containing a further O, S or NR^{14} group where R^{14} is hydrogen or C_1-C_6, alkyl;

provided that:

- when R is thiophene, furan or pyrrole then R^1 is not 4-pyridinyl or 3-pyrazolyl.
- when R is oxazole, thiazole or imidazoles then R^1 is not 3-pyridinyl and 5-pyrimidyl.

2. A compound according to claim 1 in which the group R is substituted as shown below in formula (IA) where A and B are selected from CH, S, O or NR^{11} where R^{11} is hydrogen or

C_{1-6}alkyl:



(IA)

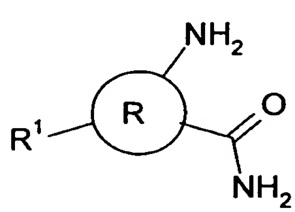
3. A compound according to claim 1 or claim 2 in which R is thiophene, furan, pyrrole, imidazole, thiazole or oxazole.

4. A compound according to any one of claims 1 to 3 in which R^1 represents optionally substituted phenyl.

A compound according to claim 1 selected from:
3-[(Aminocarbonyl)amino]-5-phenyl-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-chlorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(4-fluorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(4-methoxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-thienyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-hydroxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(2-chlorophenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(2-methoxyphenyl)-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-(3-methoxyphenyl)-2-thiophenecarboxamide,
2-[(Aminocarbonyl)amino]-5-phenyl-3-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,

3-[(Aminocarbonyl)amino]-5-{4-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{4-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{3-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-morpholinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-pyrrolidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(1-piperidinyl)ethoxy]phenyl}-2-thiophenecarboxamide,
3-[(Aminocarbonyl)amino]-5-{2-[2-(dimethylamino)propoxy]phenyl}-2-thiophenecarboxamide,
and pharmaceutically acceptable salts and solvates thereof.

5. A process for the preparation of a compound of formula (I) which comprises treatment of a compound of formula (II):



(II)

where R and R^1 are as defined in formula (I) with an isocyanate and optionally forming a pharmaceutically acceptable salt.

6. A pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

7. A process for the preparation of a pharmaceutical composition as claimed in claim 6 which comprises mixing a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 6 with a pharmaceutically acceptable adjuvant, diluent or carrier.

8. A compound of formula (I), or a pharmaceutically-acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 for use in therapy.

9. Use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4 in the manufacture of a medicament for use in therapy.

10. A method of treating an IKK2 mediated disease which comprises administering to a patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4.

11. A method of treating an inflammatory disease in a patient suffering from, or at risk of, said disease, which comprises administering to the patient a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, as claimed in any one of claims 1 to 4.

13. A method according to claim 12, wherein the disease is asthma.

ABSTRACT

The invention relates to heteroaromatic carboxamides, processes and intermediates used in their preparation, pharmaceutical compositions containing them and their use in therapy.